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ICI 182,780 Stimulates the Growth of a Uterine Cell Line Derived from p53-Deficient Mice

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ABSTRACT—UE8 is a uterine epithelial cell line established from p53-deficient fetal female mice. UE8 exhibits a typical epithelial morphology in culture and is strongly positive for cytokeratin, but negative for vimentin in immunocytochemistry. UE8 shows an active growth in a phenol red free 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s nutrient mixture F-12 supplemented with 3% heat-inactivated and dextran-coated charcoal-treated fetal calf serum. Both immunocytochemistry and immunoblot analyses confirmed that UE8 was negative for the estrogen receptor. Diethylstilbestrol added to the medium at concentrations between 10^-6 and 10^-8 M had no significant effect on the proliferative rate, and estradiol-17β at 10^-6 M was slightly inhibitory. Unexpectedly, however, the “pure anti-estrogen” ICI 182,780 at 10^-6 M significantly enhanced cell proliferation. This is the first observation that the “pure anti-estrogen” ICI 182,780 stimulates the growth of uterine epithelial cells without estrogen receptors.

INTRODUCTION

The development of pure anti-estrogens without estrogenic activity may be important in the relief of a variety of clinical problems. For this purpose, suitable in vitro models are needed to estimate the quality of the proposed agents. MCF-7 (Osborne et al., 1987) and Ishikawa (Nishida et al., 1985) cells, whose proliferation was stimulated by estrogen, have been utilized to estimate the quality of anti-estrogens with conflicting results. For example, tamoxifen is an antagonist for the growth of MCF-7 cells (Katzenellenbogen et al., 1987; Wakeling et al., 1989; Pratt and Pollak, 1993), but it is a full agonist for the growth of Ishikawa cells (Kleinman et al., 1996). Therefore, a variety of cell lines may be necessary to estimate with certainty the side effects of newly-developed reagents.

The mouse uterus is a particularly sensitive indicator of the agonist activity of anti-estrogens (Clark and Markaverich, 1982; Furr and Jordan, 1984). However, its usage is hampered by failure to establish estrogen-sensitive cell lines except for the m-M116 cells established from a leiomyoma of a CaBP9k/Tag transgenic mouse (Blin et al., 1996). Recently, we have succeeded in establishing uterine cell lines from p53-deficient mice (Hanazono et al., 1997). Throughout the characterization of these cell lines, we found that one of the lines showed a proliferative response to the pure anti-estrogen ICI 182,780.

MATERIALS AND METHODS

Cell culture and passaging

UE8 was maintained in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s nutrient mixture F-12 without phenol red (DMEM/F12; Sigma, St. Louis, MO, USA) containing 3% (v/v) heat-inactivated fetal calf serum (FCS; Commonwealth Serum Laboratories, Melbourne, Australia), supplemented with penicillin (31 µg/ml, Sigma), streptomycin (50 µg/ml, Sigma) and Fungizone (2.5 µg/ml, Life Technologies, Grand Island, New York, USA). When cells became confluent, they were washed with Ca2+- and Mg2+-free phosphate-buffered saline (PBS) and then incubated in trypsin solution at room temperature for 2 min. After the incubation, cells were removed from dishes by pipetting, and one-fortieth of them were passaged. They were continually cultured and passaged more than 140 times. Cells used in the present study were at passage between 140 and 160.

Immunocytochemical staining

The method of immunocytochemical staining was described previously (Hanazono et al., 1997). In brief, cells in a small volume of cell suspension were seeded on Lab Tek tissue culture chamber slides (Miles Labs., Naperville, IL, USA) and incubated in the medium with estradiol-17β (E2, 10^-6 M) for 3 days. Cells grown on the slides were fixed in 95% (v/v) ethanol containing 1% (v/v) heat-inactivated fetal calf serum (FCS; Commonwealth Serum Laboratories, Melbourne, Australia), supplemented with penicillin (31 µg/ml, Sigma), streptomycin (50 µg/ml, Sigma) and Fungizone (2.5 µg/ml, Life Technologies, Grand Island, New York, USA). Cells were then rinsed with ethanol (99%; v/v), and the slides were placed in 95% ethanol and then immersed in PBS.

After pre-incubation in PBS containing 5% (v/v) normal goat serum (NGS; Zymed Lab., San Francisco, CA, USA) and 1% (w/v) bovine serum albumin (BSA; Sigma) to block non-specific binding of
homogenate was sonicated for 10 sec and immediately incubated at 0°C for 20 min before collecting the lysate.

An adult CD-1 female mouse was killed by cervical dislocation and the uterus were dissected out. The tissues were minced and suspended in 1 ml 0.08 M Tris buffer (pH 6.9) containing 0.11 M SDS and 0.1% (v/v) TritonX-100 (Sigma), 1% (w/v) Na-deoxycholate (Sigma) and 0.1% (w/v) sodium dodecyl sulfate (SDS; Sigma) were added to the tube and incubated at 0°C for 15 min. The homogenate was sonicated for 10 sec and immediately incubated at 95°C for 5 min. The homogenate was centrifuged for 30 min at 2 × 10^5 × g and the supernatant was collected. Protein concentration of the samples for blotting analysis was determined using BioRad Protein Assay (Bio-Rad, Richmond, CA, USA). Aliquots of all samples were stored at –80°C until analysis.

Analysis of blotting

The samples were electrophoresed on SDS-polyacrylamide-slab gel and electrophoretically transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). Membranes were rinsed in Tris-buffered saline (TBS; pH 7.6) for 15 min. The membranes were blocked with 10% (w/v) skimmed milk (Yukijirushi-Nyuugyou Co., Sapporo Japan) in TBS-0.05% (v/v) Tween 20 (TBS-T) at 4°C for overnight and incubated with anti-ER antibody (1/50; NeoMarkers). The antibody was diluted with 5% (w/v) skimmed milk in TBS-T, which was also used to wash the membranes. They were incubated with horseradish peroxidase-conjugated antibody (Biosource) for 1 hr. Then they were washed twice with TBS-T containing 5% skimmed milk and once with TBS-T, and reacted with an enhanced chemiluminescence detection regent (ECL; Amersham, Buckinghamshire, England) at room temperature for 1 min. Autoraphs of chemiluminescence were prepared by exposing the membranes for 30 sec to X-ray film (Kodak) at room temperature.

RESULTS

Sensitivity to estrogens and detection of ER

To examine the effect of estrogens on UE8 cell proliferation, growth rates were monitored in 3% DCCFCS with increasing concentrations of E_2 or DES. As shown in Fig. 1, these estrogens had no stimulating effect on UE8 cells. E_2 slightly inhibited cell proliferation at 10^{-6} M.

Immunocytochemistry did not reveal ER in UE8 cells (data not shown), and this result was further confirmed by Western blotting. An intact mouse uterus as a positive control contained ER as a strong single band at 66 kDa. (Fig. 2, lane a), but extract of UE8 cells did not contain any detectable immunoreactive band (Fig. 2, lane b).

![Fig. 1. Estrogen sensitivity of UE8. To determine estrogen stimulation of UE8 cell proliferation, growth rates were monitored in DMEM/F12 containing 3% DCCFCS. Cells were cultured for 3 days with increasing concentrations of E_2 or DES (10^{-8}, 10^{-7} and 10^{-6} M). Control cultures (C) were grown in medium with ethanol vehicle (0.01%). Cell numbers per well were estimated by alamar Blue assay. Data are expressed as % of control growth (the mean ± SEM; n = 5). ∗, p < 0.01 vs. control.](https://bioone.org/journals/Zoological-Science/10.1093/zsc/89.5.589)
Cells were plated on 96-well dishes (5 × 10^3 cells/well) in DMEM/F12 containing 3% DCCFCS. ICI 182,780 was added at 10^{-6} M (solid bars) or 10^{-7} M (striped bars). Control cultures (open bars) were grown in medium with ethanol vehicle (0.01%). Media were changed every 2 days. At 1, 3, 5 days, cell numbers per well were estimated by alamar Blue assay. Data are expressed as % of day 1 control growth (mean ± SEM; n = 5). ∗, p < 0.01 vs. control value for each day.

**Sensitivity to ICI 182,780**

ICI 182,780 was first tested on the cells in medium containing 3% DCCFCS. Proliferation was slightly stimulated by ICI 182,780 at 10^{-7} M: 20% over the control (p < 0.01, Fig. 1) on day 5 of culture. A higher concentration (10^{-6} M) resulted in further growth stimulation: 31% on day 1, 47% on day 3 and 45% on day 5 over controls (p < 0.01, Fig. 3).

**DISCUSSION**

The mouse uterus is a particularly sensitive indicator of agonist activity of anti-estrogens (Clark and Markaverich, 1982; Furr and Jordan, 1984) and has been widely used in vivo to estimate activity of estrogen antagonists. Establishment of cell lines from the uterus may allow to determine whether an agonist acts as a pure anti-estrogen or not. Furthermore, cell lines are critical for investigation of the molecular mechanisms of antagonist action. In the present study, we reported that the proliferation of UE8 cells was stimulated by a “pure anti-estrogen” ICI 182,780, but inhibited by E_2 at 10^{-6} M. However, both immunocytochemical and immunoblot analyses revealed no detectable ER in these cells. The same results were obtained from another line UE2 which was co-established with UE8 (data not shown). This is the first observation that the “pure anti-estrogen” ICI 182,780 can stimulate the growth of uterine cells in vitro.

Our present observations raise an interesting question: whether the proliferation of UE8 cells was stimulated through ER. The growth of ER-positive MCF-7 cells is inhibited by ICI 182,780, but that of ER-negative BT-20 cells is not affected by the anti-estrogen (Wakeling and Bowler, 1992). Although neither immunocytochemistry nor immunoblot analysis demonstrated that UE8 cells have detectable ER, it is possible that the monoclonal antibody employed in the present study might be unable to recognize ER, because the p53-deficiency may cause conformational changes due to a greatly increased rate of accumulation of genetic mutations (Kastan et al., 1992). In LNCaP prostate tumor cells, a mutated steroid receptor was reported: the cells have a point mutation in the androgen receptor at the androgen-binding domain, and their proliferation was stimulated not only by androgens but also by anti-androgens, estrogens and progesterone (Veldscholte et al., 1990, 1992). Therefore, it is possible that the anti-estrogen stimulates proliferation through mutated ER which lost immunoreactivity to the anti-ER antibody. As shown in Fig. 2, E_2 at 10^{-6} M caused growth inhibition of UE8 cells; such inhibition is also reported in primary cultures of mouse uterine epithelial cells (Uchima et al., 1991), even though DES had no effect on the proliferation.

Alternatively, the anti-estrogen (or its possible contaminants) may stimulate the proliferation of UE8 cells without involving ER; DES or its metabolites can have effects through some macromolecules of ER-negative cells (Barrett et al., 1981). ICI 182,780 or its metabolites may also stimulate the proliferation of UE8 cells through a non-ER mechanism. In addition, Kuiper et al. (1996) cloned a novel rat ER (ERβ), which has low homology of its N-terminal with that of the previously cloned ER (recently renamed ERα). They reported that MCF-7 cells do not contain ERα, whereas two types of ER (α and β) are expressed in rat and human uterus (Kuiper et al., 1997). Although additional studies are needed to clarify the mechanism how the anti-estrogen stimulates cell proliferation without the ER, UE8 is an unique cell line to investigate estrogen-regulated proliferation.

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**REFERENCES**


**Fig. 3.** Effect of ICI 182,780 in DCCFCS-supplemented medium. Cells were plated on 96-well dishes (5 × 10^3 cells/well) in DMEM/F12 containing 3% DCCFCS. ICI 182,780 was added at 10^{-6} M (solid bars) or 10^{-7} M (striped bars). Control cultures (open bars) were grown in medium with ethanol vehicle (0.01%). Media were changed every 2 days. At 1, 3, 5 days, cell numbers per well were estimated by alamar Blue assay. Data are expressed as % of day 1 control growth (mean ± SEM; n = 5). ∗, p < 0.01 vs. control value for each day.

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effects of estradiol and antiestrogens. Endocrinology 137: 2246–2253


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